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USE OF MEDICAL METERED DOSE INHALERS FOR FUNCTIONALITY TESTING OF BIOAEROSOL DETECTION AND IDENTIFICATION SYSTEMS

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Fielded instrumental bioaerosol detection and identification systems must be periodically checked to ensure they are responsive. Variants of pressurized metered dose inhalers, referred to here as bioaerosol metered dose distributors (bioMDDs), are considered for this role. BioMDDs were filled with 1,1,1,2-tetrafluoroethane (HFA134a) propellant and an ethanol suspension of either fluorescently tagged polystyrene microspheres (PSLs) or simulants for pathogenic *Bacillus anthracis* spores. For PSLs or spore aerosols with geometric mean sizes of approximately 1 µm, the geometric standard deviations of aerosols are ~1.22–1.68 µm, but the aerosols are near-monodisperse for larger sizes (3 and 5 µm PSLs). Shot weight from the metering valves and aerosol dose are constant up to approximately 170 actuations, although the dose is much more variable. Devices prepared with PSLs have a useful shelf life of at least 9 months, and those prepared with biological spores have a shelf life of at least 1 year, provided they are refrigerated. BioMDDs were used to check the triggering of two UV bioaerosol detectors, and the results were favorable. The bioMDDs appear to be suitable for functionality testing but are not applicable to general calibration of field-deployed instruments. The devices may, however, be useful for checking particle-sizing devices such as optical particle counters.

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PREFACE

The work described in this report was started in January 2009 and completed in December 2011.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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CONTENTS

FIGURES

TABLE

USE OF MEDICAL METERED DOSE INHALERS FOR FUNCTIONALITY TESTING OF BIOAEROSOL DETECTION AND IDENTIFICATION SYSTEMS

1. INTRODUCTION

Field-deployed near-real-time bioaerosol detection (e.g., fluorescent aerosol particle detectors) and identification systems (e.g., polymerase chain reaction [PCR]) must be regularly checked for functionality to determine whether the systems properly respond to bioaerosol challenges. For this purpose, "acceptable functionality" is an instrumentally provided indication of the presence of a challenge, without regard to the precision of the correlation between the magnitude of the aerosol input and the level of the instrument response. The approach considered herein for functionality testing is the use of variants of medical pressurized metered dose inhalers (pMDIs), which are normally employed as delivery devices for inhalation-effective pharmaceuticals, to provide aerosols suitable for checking instruments. In this report, the medical version is referred to as the pMDI, and the variants used for functionality testing of bioaerosol detection or identification devices are called bioaerosol metered dose distributors (bioMDDs).

The principal testing involved bioMDDs filled with a formulation of propellant and an ethanol suspension of either fluorescently tagged polystyrene microspheres (PSLs) or bacterial spores. We also performed some experiments with bioMDD devices that contained only propellant. The size distributions resulting from aerosolizing the PSLs and bacterial spores were measured, and tests were conducted to characterize the uniformity of the mass output and aerosol doses from bioMDDs, including the output variations with usage (number of actuations) and with storage time. Herein, the *shot weight* or *volumetric output* of a bioMDD refers to the total mass or volume of formulation released by actuation of the device, and *dose* refers to the mass per actuation of PSLs or the number of culturable bacterial spores per actuation, in the aerosol state after evaporation of the propellant and other volatiles (e.g., ethanol) has occurred. Also in this study, we used bioMDDs to demonstrate the functionality testing of two bioaerosol detection systems, namely, an Ultraviolet Aerodynamic Particle Sizer (UV-APS, model 3314; TSI, Inc., Shoreview, MN) and a UV trigger system for a bioaerosol identifier. In the UV trigger system, the trigger is designed to be continuously operated and to provide a startup signal to an energy consuming collector-identifier system if unusual results are detected.

1.1 Background on pMDIs

Interest in apparatus for functionality testing is focused on the pMDI concept (Figure 1), which is inexpensive, self-contained, easy to operate, lightweight, and portable. Additionally, it accommodates a variable number of doses and can be stored in any orientation without leakage. A pMDI can provide consistent doses during the life of the pressurized dispensing container (PDC) (Rubin and Fink, 2005), and the high internal pressure (on the order of several atmospheres) protects container contents against external pathogenic contamination.

Regarding testing of pMDIs containing pharmaceuticals in the particulate phase, Byron (1994) evaluated the output variability and effect of pressurized container storage orientation. Results showed consistent output from devices fitted with Bespak BK356 inverted metering

valves (Bespak Pharmaceutical, Cary, NC) if the pMDIs were shaken and primed before use, where the priming consisted of five actuations before a test sample was acquired. Also, the orientation of the pressurized containers over a 24 h storage period did not affect the output provided the pMDIs were shaken and primed before use. Similar testing was conducted by Graham et al. (1992) to determine single-actuation drug content. Output was reduced when the pressurized container was stored and not shaken before use; the degree of reduction depended on the container orientation during storage.

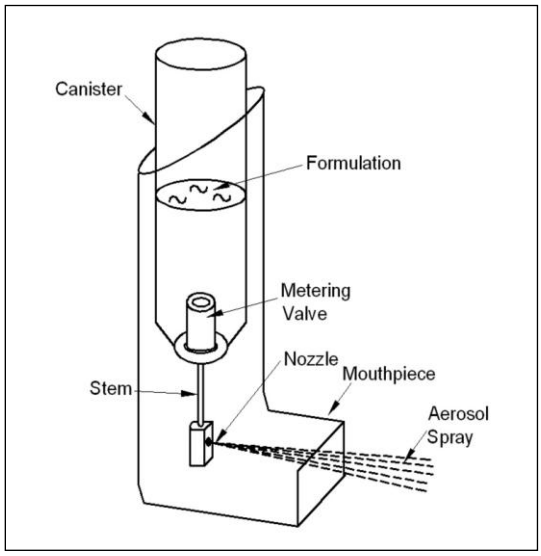

Figure 1. An illustration of a pMDI.

A medical dose inhaler is typically filled with a mixture of propellant, surfactant, preservative, and drug. Several studies have shown high variability in the metered dose released from pMDIs when solid-particle suspensions of drugs are used. Drug particles in a suspension can undergo sedimentation, so patients are instructed to shake inhalers immediately before use (Byron, 1997). The temperature of the pressurized container can affect the medication dose, with lower temperatures causing less drug to be delivered (Rubin and Fink, 2005). The volumetric output of the metering valve is generally invariant with temperature, so the observed effect is likely due to reduced vapor pressure and increased density of the propellant at lower temperatures (June et al., 1996).

The need to prime a pMDI that contains a drug suspension was also exhibited by the work of Cry et al. (1991), who showed substantially less and highly variable drug doses in the first actuation as compared with subsequent actuations. The shot weight was the same for the first, second, and third actuations, so the metering valve performed its function properly in dispensing a constant volume of liquid; however, the reduced dose from the first actuation suggests that the concentration of particulate matter in formulation released during the first actuation was less than that released in subsequent actuations.

The need to resuspend drug particles and prime a pMDI device is well established, and both procedures must be implemented in the protocol for using a bioMDD. For testing bioMDDs in the laboratory, resuspension can be accomplished with either sonication or shaking; however, for testing in the field, shaking is more practical.

1.2 Nonmedical Applications

The pMDI technology has been explored for generating nonpharmaceutical aerosols. Vervaet and Byron (2000) filled canisters of pMDIs with 1, 3, 5, and 8 µm PSLs and showed that suspensions were stable for 6 months at room conditions, but needed sonication before use. Carrera et al. (2005) prepared devices with *Bacillus atrophaeus* var. *globigii* (BG) spores, which are used as a simulant for pathogenic *Bacillus anthracis* (BA) spores because of their similar size and physical properties. Using high-resolution photography and computer-assisted imaging, they tested a method for quantifying the number of spores in bioaerosol clusters formed from atomizing BG suspensions.

The focus of this study was to determine the output quantity and consistency of bioMDDs, including devices that contained 1, 3, and 5 µm PSLs and clusters of spores of BG and *Bacillus thuringiensis* subsp. *israelensis* (Bti), where the latter organism was also used as a simulant for BA. Its innocuousness is demonstrated by its general acceptance for use as an insecticide (Schnepf et al., 1998). The effects on the Bti-filled bioMDDs of storage at room and lowered (refrigerator) temperatures and the effects on the PSL-filled bioMDDs of storage at room conditions were included in the evaluations.

2. METHODS AND MATERIALS

The canisters of bioMDDs used in this study were filled by means of a Pamasol P2005 small-scale production plant (Pamasol Willi Mäder AG, Pfäffikon, Switzerland). The aluminum canister (5.06 g) of each bioMDD was filled with 0.40 g (0.5 mL) of test materials and 11.72 g (9.55 mL) of 1,1,1,2-tetrafluoroethane (HFA134a) propellant. For the bioMDDs used to generate PSL aerosols, the formulation materials were fluorescent PSLs suspended in ethanol; the as-received PSL latex (hydrosol with 0.5% surfactant) was diluted in ethanol, centrifuged, and then resuspended in ethanol as a means of reducing the water and surfactant content of the formulation. The Bti and BG formulations were prepared in a similar manner whereby the as-received material, which was either in the hydrosol or bulk powder state, was diluted with ethanol, centrifuged, and rediluted at least one time.

The metering valves have a nominal output of 50 µL/actuation (model BK357, Bespak Pharmaceutical, Cary, NC), which ideally provide about 200 dose actuations for the approximately 10 mL fill volume of the containers. The preparation process used in this study followed that of Byron (1994). A gasketed lid, which contains the metering valve, was crimped to the filled canister to form the PDC. The stem of the metering valve protruded from the PDC (Figure 1), and movement of the stem into the valve released the valve contents.

2.1 BioMDD Output Delivery Methods

The following configured devices were employed in this experimental study (shown in Figure 2):

(a) The classical pMDI with the mouthpiece and nozzle, whereby the latter is a Bespak spray nozzle (0.50 mm port) that discharges aerosol through the mouthpiece at a right angle to the axis of the PDC.

(b) A PDC with no nozzle or actuator that releases aerosol directly from the metering valve stem, which has a 2 mm i.d.

(c) The PDC of Figure 2b fitted with the nozzle of Figure 2a.

(d) The PDC of Figure 2b with an actuator that has four right-angle, 3.2 mm diameter exhaust ports.

(e) The PDC of Figure 2b fitted with an axial flow disk actuator that is slip-fitted over the end of the 3.2 mm o.d. stem, but with the end of the stem shouldered against a 2.1 mm diameter port in the actuator (to prevent the actuator from sliding on the stem during use).

(f) The PDC of Figure 2b fitted with an actuator similar to that of Figure 2e, but with no shoulder on the actuator, so the actuator is essentially a hub on the stem (two setscrews prevent the actuator from sliding on the stem during use).

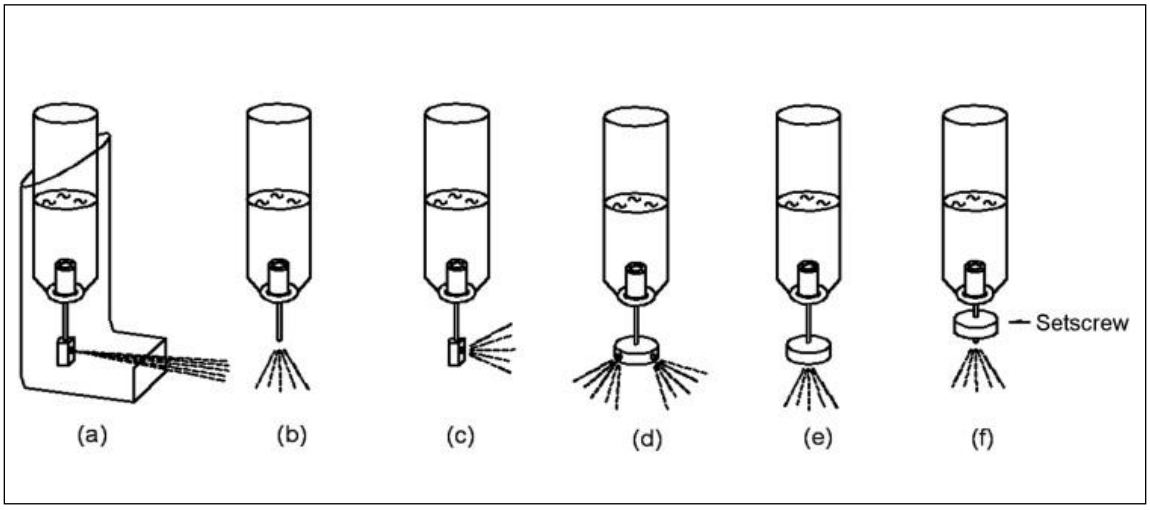

Figure 2 (a–f). Configurations of bioMDD devices used in this study: (a) a pMDI; (b) a PDC with no nozzle or actuator; (c) a PDC with a Bespak nozzle that serves as an actuator; (d) a PDC with an actuator that has four right-angle, 3.2 mm ports; (e) a PDC with an actuator that is shouldered against the stem and has an axial exhaust port; and (f) a PDC with an actuator that is slip-fitted over the stem and locked in place with a setscrew.

2.2 Size Distributions of Aerosol Particles

Output size distributions of PSL, BG, and Bti aerosols, generated using the basic pMDI configuration shown in either Figure 1 or 2a, were measured with an Aerodynamic Particle Sizer (APS, model 3321, TSI, Inc.). For some of the tests, the PDCs were ultrasonicated before the test and again just before use, and the bioMDDs were hand-shaken for 5 s and subsequently primed with three actuations. Tests were also conducted in which PDCs with some of the formulations were not ultrasonicated but were shaken before use. BioMDD output was delivered into a 118 L chamber from which samples were drawn into the APS for size measurement.

Because both bioaerosol detectors and identifiers are generally sensitive to particle mass rather than particle number, the number-size frequency data provided by the APS was converted to normalized volume distributions. The purpose of the conversion was to enable calculation of the volume in each of the APS size intervals from the number of particles and the midpoint aerodynamic diameter (AD) associated with each of the intervals. Also, the geometric means and standard deviations of the volume distributions were calculated (Hinds, 1999).

2.3 Shot Weights of BioMDDs

The mass output per actuation (shot weight) of bioMDDs without an actuator (Figure 2b) and with various actuators (Figures 2a, c, and d) was measured as a function of usage (number of actuations). The bioMDD without an actuator was filled with only propellant, whereas the formulation in the PDC of the bioMDD also included BG spores, which ideally provide 106 spores/actuation (based on colony-forming unit [cfu] concentration in the formulation and volume of the metering valve). The pressurized dispenser containers were weighed after every 10 actuations to quantify the shot weights.

2.4 Balloon Collection Method

Various methods, such as impaction, filtration, or impingement, could be considered for collecting the aerosol for analysis. Phillips et al. (1990) used impaction and impingement to collect aerosols generated from albuterol formulations, and Kamayi et al. (2004) used two types of impactors to examine aerosol size distributions. In this study, a balloon collection method was used to characterize the aerosol doses from the test devices. This method was selected to help preserve the culturability of the organisms, to capture the full aerosol plumes emitted by the devices, and to accommodate the collection of large numbers of samples during short periods of time.

A washed latex balloon was partially filled with 10 mL of either deionized water with 0.01% Triton X-100 (Sigma, St. Louis, MO) for tests with PSLs, or with phosphate-buffered saline plus 0.01% Triton X-100 (Sigma) for tests with biomaterials. The output of a bioMDD was released directly into a balloon. BioMDDs with mouth actuators (Figure 2a) were not used in tests involving the balloon method, because the backside of a mouth actuator is open to the atmosphere, which allows aerosol flow to be vented to the ambient air. However, a mouth actuator (Figure 2a) was typically used during priming of a bioMDD, whereby a device was shaken for 5 s and then actuated three times before the aerosol was released into a balloon.

After the bioMDD output was discharged into the balloon, the balloon was shaken for 5 s and disconnected from the bioMDD. It was then allowed to sit for 20 min to permit the aerosol particles to sediment to a surface. After the 20 min period, the balloon contents were vigorously mixed, and the hydrosol was transferred to a 50 mL conical tube for analysis. Fluorescent PSLs were quantified by fluorometry, and bioparticles were quantified by culturing.

Validation tests were conducted to determine the recovery of 1, 3, and 5 µm PSLs and Bti spores from balloons. Evaluation of the results indicated the recovery to be 93 ± 2%, 91 ± 4%, and 86 ± 7% for the 1, 3, and 5 µm PSL suspensions, and 84 ± 11% for the Bti suspensions (values after the ± symbols are standard deviations). Herein, the recovery value used for PSLs is 90% (the average of the three sizes); for the biological spore aerosols, the recovery value is 84%.

2.5 Fluorescence Analysis of PSLs

PSLs with green fluorescent dye (Duke Scientific Corp., Palo Alto, CA) were used in the experiments with the balloon collection method. The hydrosol from the balloon was analyzed with a fluorometer (model 450, Sequoia-Turner, Mountain View, CA) that was fitted with an NB460 excitation filter and an SC500 emission filter. Approximately 3 mL of hydrosol was used in each fluorometric analysis.

2.6 Dose Ratio

Some of the results of this study are presented in terms of a dose ratio, R_d, which is the measured dose per actuation divided by the calculated dose per actuation, whereby the latter is based on the amount of material filled into the canister. For tests with PSLs, the dose ratio is calculated from

$$R_d = \frac{m_b}{\eta_b c_{m,0} V_{mv} N} \tag{1}$$

where m_b is the aerosol mass recovered from the balloon (in relative fluorometric units), η_b is the particle collection efficiency of the balloon method, $c_{m,0}$ is the initial PSL mass concentration in the pressurized dispersing container (in relative fluorometric units per volume), V_{mv} is the liquid volume per actuation dispensed by the metering valve (assumed to be 50 µL/actuation), and N is the number of actuations during a test.

2.7 Constancy of PSL Dose with Use

Experiments were conducted with formulations of suspensions of the nominally sized 1, 3, and 5 µm PSLs to determine if PSL doses were constant with actuator usage. PDCs containing the 1, 3, and 5 um PSLs had been filled 1.9, 2, and 4.9 months, respectively, before testing began.

The configuration of the bioMDDs used in these experiments is shown in Figure 2b. The balloon method was used to collect the PSLs, which were then analyzed fluorometrically. Actuations were accomplished by pushing the stem, which was inside the balloon, against a hard surface placed at an angle to the balloon. Only a small opening was needed for the output to be

delivered into the balloon. In comparison with actuation using the mouthpiece and nozzle (0.50 mm diameter) in the Figure 2a configuration, the procedure of pushing the stem, which has a 2 mm i.d., at an angle against a hard surface was expected to provide a larger opening.

The PDCs for the 1 μm PSLs were hand-shaken before use, whereas those for the 3 and 5 μm PSLs were sonicated before use. For each group of 10 actuations with the 1 and 3 μm PSLs, the bioMDDs were shaken and primed three times, five actuations were performed into the balloon, and two subsequent actuations were discarded. A similar approach was used for the 5 μm PSLs, except balloon collection was performed only on every other group of 10 actuations. Duplicate tests were conducted for each particle size through use of two similarly filled PDCs for each particle size. Results are presented in terms of dose ratios.

2.8 Effect of Storage Time on PSL Dose Output

The dose ratios of bioMDDs containing 1, 3, and 5 μm PSLs were characterized as a function of storage time. The bioMDDs used in these tests were configured as shown in Figure 2b, and aerosols were collected using the balloon method. Tests were conducted 3, 5, and 9 months after the canisters were initially filled; all PDCs were used after being stored. Immediately before testing, each PDC was sonicated for 5 min, hand-shaken for 5 s, and then primed.

2.9 Culture Analysis

Classical microbiological methods were used to quantify the colony-forming units associated with Bti and BG tests. The hydrosol from a balloon was transferred to a 50 mL conical tube, which was shaken on a vortexer (VWR International, Thorofare, NJ) for a few seconds. Either 100 or 200 μL of hydrosol was plated onto 100 mm tryptose agar plates and incubated at 37 °C for 12–16 h. After incubation, the colony-forming units were counted.

2.10 Spore Dose Variability and Effect of Actuator Use

Eight bioMDDs, which were filled with a Bti formulation that ideally provides 10^4 cfu/actuation, were used in a set of tests to determine the dose variability and the effect of the presence of an actuator. Four bioMDDs were configured with no actuators (Figure 2b) and four were configured with axial-type actuators (Figure 2e). Aerosol output of the bioMDDs was collected using the balloon method. Either 19 or 20 samples, with 2 actuations per sample, were collected from each bioMDD. All samples were analyzed via classical microbiological culturing.

A second set of tests was conducted in which bioMDDs containing BG formulation were tested with three types of actuation approaches: no actuator (Figure 2b), a hub-type actuator that allows flow to be vented directly from the stem (Figure 2f), and a nozzle-type actuator (Figure 2c). These tests were intended to clarify whether the hub- and nozzle-type actuators have any pronounced effect on aerosol dose. Results are presented in terms of colony-forming units per actuation as a function of actuation number, as reliable data were not available on the initial colony-forming unit contents of the PDCs. However, all PDCs of all bioMDDs had been filled with the same formulation.

2.11 Effects of Storage Time and Temperature on Bti Output

Fourteen Bti-filled bioMDDs (ideal initial dose,10^4 cfu/actuation) were used in these tests. The PDCs of seven bioMDDs were stored in a refrigerator (4 °C), and the other seven were stored at laboratory temperature (about 24 °C). Tests were conducted during the period of 9 to 25 months after the bioMDDs were filled, using the balloon collection method, and results were analyzed using classical microbiological culturing. The bioMDDs were operated without actuators in the configuration shown in Figure 2b.

Storage results are represented as the biological equivalent of the PSL dose ratio (eq 1). Here, the biological dose ratio, $R_{d,bio}$, is defined as

$$R_{d,bio} = \frac{CFU_b}{\eta_{b,bio}\, c_{CFU,0}\, V_{mv}\, N} \tag{2}$$

where CFU_b is the number of culturable cells recovered per actuation, $\eta_{b,bio}$ is the recovery of spores using the balloon method (84%), and $c_{CFU,0}$ is the concentration of culturable cells in the PDC at time zero.

3. RESULTS

3.1 Aerosol Particle Size from APS Measurements

The geometric mean of the number distribution and the geometric mean and standard deviation of the volume distribution for the nominal 1, 3, and 5 µm PSL aerosol particles and BG and Bti spores are presented in the Table. For the nominal 1, 3, and 5 µm PSLs, which had undergone sonication before aerosolization, the geometric volume means were 1.40, 3.25, and 5.35 µm AD, respectively, and the corresponding geometric standard deviations were 1.68, 1.13, and 1.04, respectively. For the bacterial spores generated subsequent to sonication, the geometric means and geometric standard deviations were 0.75 µm and 1.22, respectively, for BG spores, and 0.87 µm and 1.46, respectively, for Bti spores. The geometric standard deviations of the nominal 1 µm PSLs and the bioaerosols were higher than those of the nominal 3 and 5 µm PSL aerosols. These results suggest the presence of trace amounts of nonvolatile compounds (e.g., surfactant) in the PSL formulations, which produce residual droplets on the same order in size as the 1 µm PSLs or the bioparticles and tend to increase the dispersion of the aerosol.

Table. Size Characteristics of Test Aerosols. The bioMDD of Figure 2a was used to generate the aerosols.

Aerosol		Deagglomeration Technique	Geometric Number Mean (μm AD)	Geometric Volume Mean (μm AD)	Geometric Standard Deviation of Volume Distribution
PSL	1 μm	Shaken	0.81	1.54	1.59
	1 μm	Sonicated	0.76	1.40	1.68
	3 μm	Sonicated	2.77	3.25	1.13
	5 μm	Shaken	5.26	5.35	1.04
BG		Sonicated	0.70	0.75	1.22
Bti		Shaken	0.72	0.89	1.51
		Sonicated	0.68	0.87	1.46

When using the APS, it is customary to select the range of particle sizes to be included in the analyses. For larger particles, we truncated the analysis range at the upper and lower size bins that contained less than 0.1% of the total counts; this effectively eliminated the small-sized background particles. For the micrometer- and smaller-sized particles, the upper analysis range could be truncated, but not the lower, as there were still significant counts in the smallest size bin. This approach had the effect of reducing the geometric standard deviations of the larger aerosol size distributions, but it did not significantly affect the smaller size distributions.

To determine whether such residual particles result from leaching of fluorescent dye by the propellant or alcohol during storage, tests were conducted whereby aerosols generated from bioMDDs containing 2 and 3 μm fluorescently tagged PSLs were analyzed with a UV-APS (model 3314, TSI Inc.). The device provides data on both the number-size distribution of an aerosol and the fluorescence of individual particles as a function of size. The results showed the presence of residual particles; however, they were not fluorescent, which implies that leaching of the fluorescent tracer in the PSL was not significant.

Tests were also conducted in which PDCs containing 1 μm PSLs and the Bti formulation were shaken, but not sonicated, before use. Those results (Table) suggest that the sonication did not greatly affect the volume median size of the resulting aerosol. The geometric volume mean ADs of the 1 μm PSLs and Bti aerosols were 1.40 and 0.87 μm, respectively, when the PDCs were first sonicated and then shaken immediately before use, as compared with 1.54 and 0.89 μm, respectively, when the PDCs were only shaken.

If the geometric standard deviation of an aerosol is small (e.g., for 5 μm PSLs, the value was 1.04 [Table]), the geometric means of the number and volume distributions will be approximately the same. However, for larger values of the geometric standard deviation, the geometric mean of the volume distribution can be considerably larger than that of the number distribution. For the 1 μm PSLs (Table) that were sonicated before use, the number and volume geometric means were 0.76 and 1.40 μm AD, respectively. Ostensibly, the number geometric mean AD was less than the physical size of ~1 because of the presence of trace amounts of nonvolatile contaminants in the formulation.

3.2 Shot Weight Constancy of BioMDDs

Shot weights (as measured by changes in PDC weight per actuation) of a bioMDD filled with only propellant and a bioMDD filled with BG formulation are shown in Figure 3. Stable outputs were obtained with either approach up to approximately 170 actuations, above which shot weights declined significantly. The two configurations had slightly different mass outputs. The bioMDD rendition with propellant alone produced shot weights of 61.2 ± 1.3 mg/actuation for the first 170 actuations, whereas the bioMDD with BG produced shot weights of 57.0 ± 1.1 mg/actuation for the corresponding range of actuations. The use of different actuators had little effect: the mass output of the bioMDD fitted with the actuator arrangement of Figure 2a was 56.5 mg/actuation, that of Figure 2c was 58 mg/actuation, and that of Figure 2d was 56.9 mg/actuation.

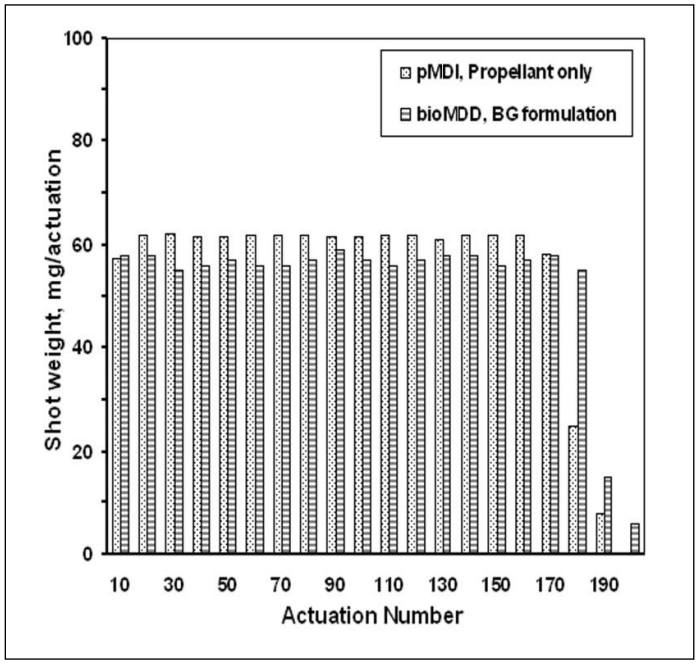

Figure 3. Mass output per actuation from a bioMDD containing propellant only and a bioMDD containing BG formulation. The latter device was fitted with various actuators (as shown in Figures 2a, 2c, and 2d) during the testing.

The metering valve essentially controls the formulation output volume during each actuation from the PDC of a bioMDD. There was a slight difference in the densities of propellant (1226 kg/m^3) and BG formulation (1212 kg/m^3). For these two density values, the bioMDD filled with propellant only released an average of 49.9 ± 1.1 µL/actuation, and that filled with BG formulation released an average of 47.0 ± 0.9 µL/actuation. A statistical *t* test infers, at the 95% confidence level, that there is a difference between the two data sets (p = 8.5E-10). However, the U.S. Food and Drug Administration (FDA; 1998) considered providing guidelines for metered dose inhalers that included considerations of metering valve performance. It was suggested that the shot weight of acceptable individual delivery valves should be within 15% of the desired

value. Assuming the desired volumetric output is 50 µL/actuation, the values of 49.9 and 47.0 µL/actuation were acceptable. Although those FDA guidelines were not implemented, they do provide an indication of desired performance of metering valves. As is shown later, this difference between the two data sets is small when compared with other effects.

Possible changes in shot weight for 170 useful actuations of the devices can be further explored statistically. When linear regression lines are fit through the two data sets (mass output per actuation vs. actuation number) the resulting slopes provide indications of the change in output with use. For both data sets, statistical *t* tests at the 95% confidence level accept a null hypotheses that the slopes of the lines are zero, which implies the outputs of both the bioMDD configuration with propellant only and the configurations with BG formulation are constant with usage.

3.3 Effect of Number of Actuations on PSL Dose Output

BioMDD devices configured as shown in Figure 2b were used to test the dose constancy for 1, 3, and 5 µm PSLs. With reference to Figure 4, qualitatively it appears that approximately constant dose ratios were obtained with up to 170 actuations for the three particle sizes, although actuation-to-actuation variations were much greater than those for the shot weights (Figure 3).

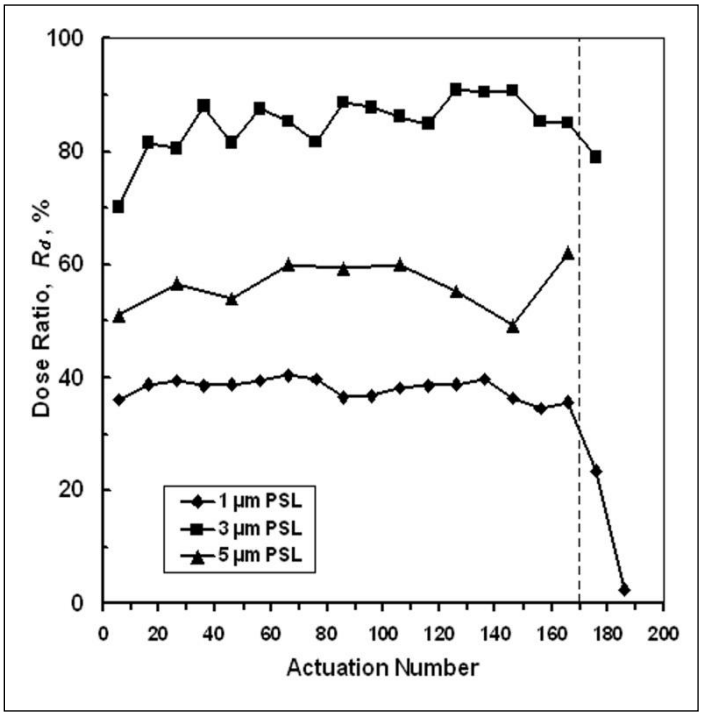

Figure 4. Constancy of PSL doses with usage. Dose ratio is the actual mass of aerosol particles released per actuation divided by the calculated amount released per actuation. BioMDDs were used without actuators (configuration shown in Figure 2b). A dashed line is shown at 170 actuations, which represents the approximate limit of usefulness of a 10 mL canister fitted with a 50 µL/actuation metering valve.

For the 1, 3, and 5 µm PSLs, the average dose ratios for the regions associated with ≤170 actuations were 38.0 ± 1.7%, 85.1 ± 5.0%, and 56.4 ± 4.4%, respectively. The slopes of the lines analyzed with *t* tests yielded values that were not different from zero for 1 and 5 µm PSL bioMDDs, with *p* values of 0.10 and 0.51, respectively; however, that of the 3 µm PSL bioMDD was significantly different from zero ($p = 0.007$).

The canisters for these tests had been filled 2 to 5 months before the tests were conducted, and as noted (section 3.4), storage time affects PSL dose ratio. The lower dose ratio values for the 1 µm PSL particles were likely a result of the greater adherence of those particles to the inner walls of the PDC. Kesavan et al. (2010) observed a similar phenomenon with 1 µm PSLs in bioaerosol samplers that collected time-integrated hydrosol samples.

3.4 Effect of Storage Time on PSL Dose

After storage periods of 3, 5, and 9 months, the dose output from bioMDDs filled with 1, 3, and 5 µm PSLs were measured using the balloon collection method and subsequent fluorescence analysis of the collected aerosol mass. The results, including the mean dose ratios and associated error bars for each size and storage period, are shown in Figure 5. The average dose ratios over all storage times were 18, 64, and 78% for the 1, 3, and 5 µm PSL sizes, respectively. Again, the considerably lower dose ratio values observed for the 1 µm AD particles were likely due to greater adhesion of the smaller particles to the internal walls of the canisters even though all canisters were sonicated for 5 min before use. Higher dose ratios were observed for the samples evaluated at 5 months of storage, which could be the result of more effective sonication of those samples. A comparison of the samples stored for 3 and 9 months reveals the error bars for a given size at the two storage times overlap, which suggests that statistically, the results would be indistinguishable.

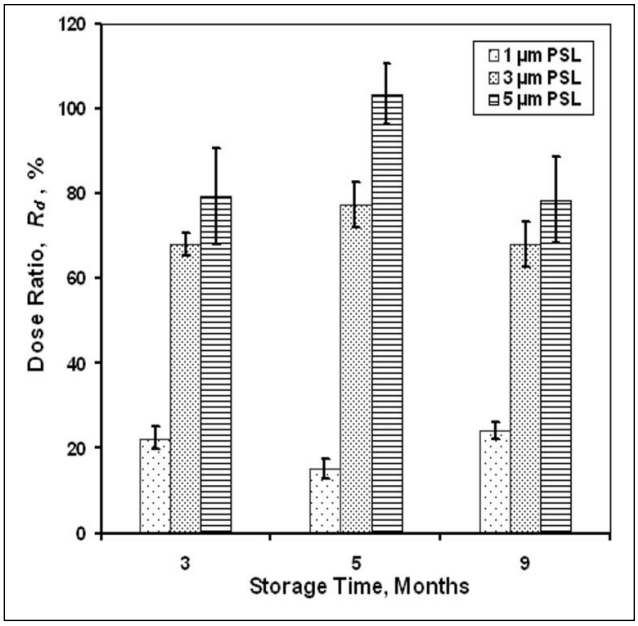

Figure 5. Effects of storage time on dose ratio for PSL-containing bioMDDs. BioMDD configuration used for the aerosol releases is shown in Figure 2b.

With reference to Figure 4, where the dose ratio is shown as a function of the number of actuations, the canisters had been filled 2 to 5 months before testing, so the two experiments can be compared in an approximate manner. As shown in Figure 4, the dose ratio values were 38% for the bioMDD containing 1 µm PSLs that was prepared 1.9 months before testing; 85% for the bioMDD containing 3 µm PSLs that was prepared 2 months before testing, and 56% for the bioMDD containing 5 µm PSLs that was prepared 5 months before testing. The average values for the three storage periods (shown in Figure 5) were 18% for the 1 µm size, 64% for the 3 µm size, and 78% for the 5 µm size. These differences suggest that quantitative doses, such as those needed to calibrate instruments, are not presently obtainable with the bioMDDs. However, even after storage for 9 months, the devices yielded PSL dose ratios on the order of 18% or greater, which should be sufficient to check instrument functionality provided the initial concentration of particulate matter in the formulation was suitable.

3.5 Bti and BG Spore Doses

3.5.1 Effect of Actuator Use and Constancy of Output with Bti

The results of tests with eight bioMDDs prepared with Bti formulation are as shown in Figure 6. Four of the bioMDDs were configured with shouldered axial actuators (Figure 2e) and four were configured with no actuators (Figure 2b). Testing involved collecting and culturing the aerosol generated from 2 actuations out of each group of 10 actuations; therefore, from a total of 170 actuations of each bioMDD, 17 samples comprising 34 actuations were analyzed.

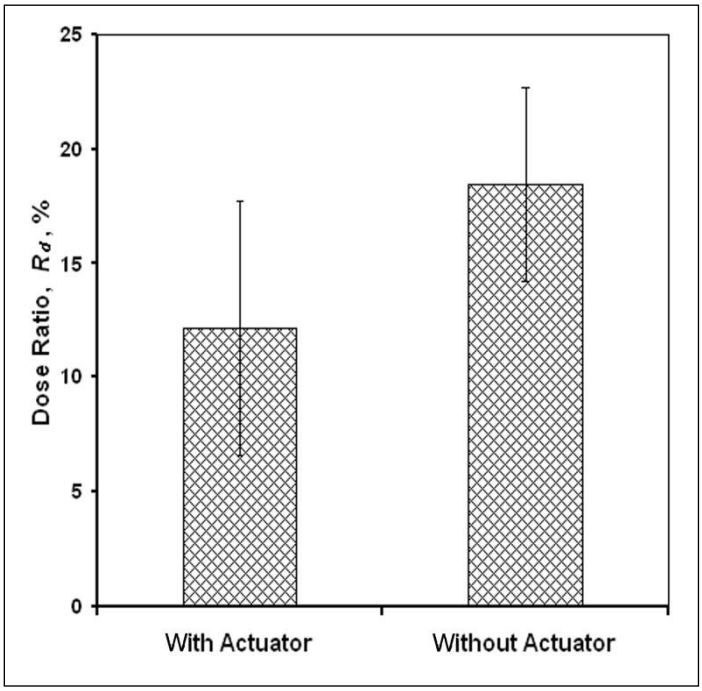

Figure 6. Bti dose ratios for bioMDDs fitted with shouldered axial actuators (Figure 2e) and no actuators (Figure 2b). For each bioMDD, 170 actuations were performed: samples were analyzed from 2 out of each group of 10 actuations.

For the four bioMDDs with shouldered axial actuators, the average dose ratio was 12.1 ± 5.6%, whereas the average for tests with no actuators was 18.4 ± 4.2%. A statistical *t* test shows these values to be significantly different ($p < 6.2E-12$). These results imply that the use of an actuator of the design shown in Figure 2e reduced the output of a bioMDD. It appears that at least part of the dose reduction was caused by deposits on the actuator exhaust port, which were observed subsequent to the testing.

Because four bioMDD units were used in the testing, the results shown in Figure 6 can be used to test a null statistical hypothesis that there is no significant difference between replicate devices. For the four units without actuators, an analysis of variance (ANOVA) at the 95% confidence level accepts the null hypothesis ($p = 0.16$). However, results of ANOVA of the bioMDDs with the disk actuators suggest that there is a difference in the output between the units ($p = 8.25E-5$).

With respect to the constancy of dose with use, Figure 7 shows a plot of the dose ratio as a function of actuation number for the bioMDDs without an actuator (Figure 2b), which showed the greatest scatter between doses. A statistical *t* test at the 95% confidence level accepted a null hypothesis that the slope of a regression line (dose ratio vs. actuation number) was zero ($p = 0.202$). The coefficient of variation (CV; the ratio of the standard deviation to the mean) of dose ratio values for the data set comprised of less than 170 actuations was 24%.

3.5.2. Replicate Canisters and Different Actuators with BG

A second set of tests conducted with BG was intended to determine whether dose was affected by use of bioMDDs with no actuator (Figure 2b), with a hub-type actuator (Figure 2e), or with a nozzle-type actuator (Figure 2c). Four replicate PDCs were used for each actuation approach; therefore, a total of 12 data sets were collected. Regarding dose constancy, at the 95% confidence level, none of the data sets exhibited statistically significant dose variations with actuation numbers up to the maximum of 170 actuations.

The mean dose per actuation (over 170 actuations) for the three actuation approaches and four replicate PDCs are shown in Figure 8. Qualitatively, the plot suggests that the average dose was affected by actuator type and replicate PDC. ANOVA was used to test a null hypothesis of equal doses from replicate PDCs. At the 95% confidence level, the null hypothesis was accepted for the devices fitted with nozzle-type actuators but rejected for the hub-type actuators and the devices with no actuators. ANOVA was then used to test a null hypothesis that there is no significant difference between actuation approaches. At the 95% confidence level, the hypothesis was rejected when comparing the shoulder-type with the nozzle-type actuator and the shoulder-type actuator with the devices fitted with no actuators.

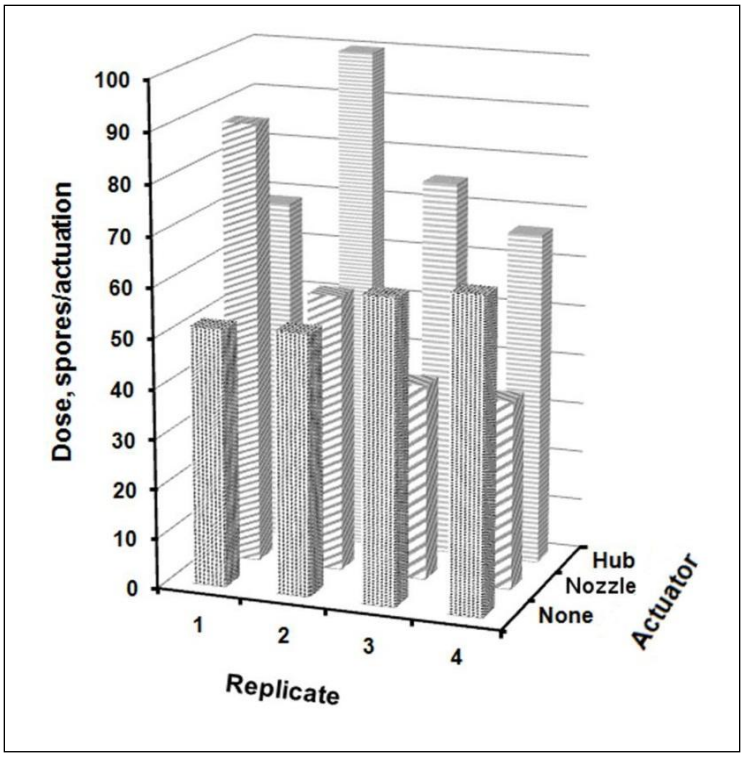

Figure 7. Dose of BG spores for three actuation approaches (Figures 2b, c, and e) with four replicate PDCs for each actuator type. Data in each set represent the average for a set of 170 actuations.

These results do not provide a basis for a recommendation on actuator choice. In Figure 8, the average dose was greatest from the four PDCs fitted with hub-type actuators, and the variation with replicate PDCs was the least for the PDCs fitted with nozzle-type actuators. As a consequence, the actuation approach could be selected on the basis of accommodating the practical aspects of providing doses to a detector or identifier, which would generally suggest either a nozzle- or hub-type actuator would be most suitable.

For the results shown in Figure 8, the overall average for the 168 tests was 65 culturable spores/actuation, and the root mean square error, exclusive of effects of actuator type, was 24 spores/actuation, which suggests the CV for BG doses was approximately 37%. In general, such a CV would make these bioMDDs unsuitable for instrument calibration.

3.6 Effects of Storage Time and Temperature on Bti Dose

It is desired that spore-filled bioMDDs, which are used to check the functionality of bioaerosol detectors and identifiers, should have a useful shelf life of 1 year or more. To determine whether storage time causes a loss of usefulness, PDCs were filled with Bti formulations and then stored either in a refrigerator (4 °C) or at room temperature (24 °C) for 9 to 25 months. Aerosol outputs from the devices were tested by culturing spores collected using the balloon method.

The resulting bioaerosol dose ratios are shown as a function of storage time in Figure 9. At the end of a 9 month storage period, the dose ratios were 64.8 and 71.2% for the samples stored at 24 and 4 °C, respectively. Statistically (at the 95% confidence level), after 9 months of storage, there was no significant difference between the room temperature and refrigerated samples. On the other hand, at the end of 25 months of storage, there was an order of magnitude greater loss in bioaerosol dose ratio for the samples stored at room temperature. Also, 1 year after the samples were refrigerated at 4 °C, the dose ratio was still 32%, which implies that if the PDC were filled with a formulation that ideally provides 10^4 spores/actuation, the actual dose per actuation would be ~3000 spores.

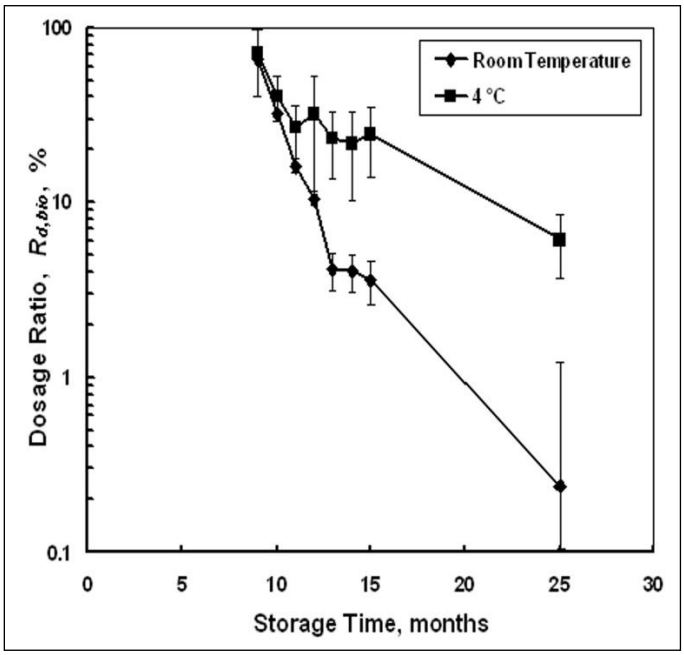

Figure 8. Effects of storage time and temperature on output of bioMDDs with Bti formulation. Dose ratio is the number of culturable cells recovered (using balloon-method collection) divided by the predicted number of culturable cells based on culturability at time zero. Room storage temperature was approximately 24 °C.

3.7 Demonstrations of Bioaerosol Detector Functionality Testing

The TSI UV-APS is designed to be used as either a standalone bioaerosol detector or as a trigger for a bioaerosol identifier (e.g., a PCR analyzer). A test was performed using a bioMDD fitted in the configuration of Figure 2a that had been prepared 3 years earlier with a formulation containing fluorescent 1 µm PSLs. The bioMDD was actuated to release 1 dose into a 32 L box. After the aerosol was mixed, it was sampled with the UV-APS. When the bioMDD was freshly filled, the formulation concentration would have produced an ideal dose of 10^6 particles/actuation. The UV-APS detected 1622 particles in a 20 s period, which is equivalent to a dose ratio of about 16%. This dose ratio is comparable to the 18% value observed for 1 µm PSLs after 9 months of storage (Figure 5), which suggests that the PSL-filled bioMDDs may have a shelf life well beyond 1 year.

An 80 L/min bioaerosol detector that also uses UV detection and serves as a trigger for a power-intensive identification system was tested in which doses from bioMDDs were drawn directly into the sampler inlet. The bioMDDs, which were in the configuration shown in Figure 2a, had been prepared with BG, γ-irradiation–killed BG, DNA from BG, Bti, ovalbumin, and 1, 2, 3, and 5 µm PSLs. The tests showed the trigger provided alarms for BG, γ-irradiation–killed BG, DNA from BG, and some PSLs. It is likely that optimized doses (higher formulation concentrations or multiple doses) would have also caused alarms for the other aerosolized materials.

4. SUMMARY AND DISCUSSION

4.1 Aerosol Size Distributions

The size distributions generated from bioMDDs filled with formulations of ethanol suspensions of 1, 3, and 5 µm PSLs, Bti spores, and BG spores in HFA134a propellant were determined with the aid of an APS. The number-size distributions measured with the APS were converted to the equivalent of volume-size distributions. The volume geometric means of the 1, 3, and 5 µm PSLs were approximately 1.5, 3.3, and 5.4 µm AD, respectively, whereas those of the Bti and BG spores were 0.8 and 0.9 µm AD, respectively. Geometric standard deviations of the 1 µm PSLs and the spore aerosols were larger than those of the 3 and 5 µm PSLs, presumably because of the presence of trace amounts of nonvolatile compounds in the formulations, which produce small-sized residual particles upon evaporation of spray droplets that do not contain the basic test particles. These small amounts of contamination could also cause a downward shift in the APS-measured size distributions of the test aerosols. The geometric means of the number- and volume-size distributions agreed well when the geometric standard deviation was small; for example, for the 5 µm PSLs, for which the geometric standard deviation was 1.04, the geometric means were 5.26 and 5.35 µm AD for the number- and volume-size distributions, respectively. However, agreement was worse for the smaller-sized particles, where the geometric standard deviations were larger. As an example, the 1 µm PSLs, which had been sonicated before use, had a geometric standard deviation of 1.68, and the corresponding geometric means of the number and volume distributions were 0.76 and 1.40 µm AD, respectively. Although the aerosol sizes are not precisely the same as those of the original particles, it is possible that bioMDD devices filled with PSL formulations could be used in field tests of size-measurement instruments to check the indicated particle sizes.

4.2 Shot Weights

Metering valves used in the bioMDDs had a nominal output of 50 µL/actuation. Tests with bioMDDs filled with either propellant only or BG formulation showed that the metering valves provided constant shot weights, as calculated from weight changes of the PDCs, up to about 170 actuations. In terms of volume, this corresponds to an ~8.5 mL discharge from a 10 mL PDC. Volumetric output of the bioMDDs filled with propellant only was slightly higher than that for the bioMDDs that contained BG suspension (49.9 vs. 46.9 µL/actuation).

4.3 PSL Doses: Constancy and Effect of Storage

Tests were also conducted with bioMDDs prepared with PSL formulations to determine the constancy of dose with usage (number of actuations). The doses were constant for <170 actuations; however, the dose ratios for the 1, 3, and 5 µm PSLs were 38.0, 85.1, and 56.4%, which also puts application of the bioMDDs outside the realm of use for quantitative instrument calibration. Dose ratios were determined for bioMDDs containing PSLs after storage for periods of 3, 5, and 9 months. There were no major changes in dose ratio with storage time, which suggests that the shelf life of bioMDDs filled with 1, 3, or 5 µm PSLs was at least 9 months. In a subsequent test in which the output of a PSL-filled bioMDD that had been stored for 3 years was sampled with a TSI UV-APS, the dose ratio was 16%. This suggests that useful outputs of stored bioMDDs may be realized well beyond the 9 month time period.

4.4 *Bacillus* Spore Aerosols: Variability Considerations and Effects of Actuator Type and Storage Time

Experiments were conducted with Bti spores and bioMDDs either with shouldered axial flow actuators or without actuators. Dose ratios were 12.1 and 18.4% for the two data sets, respectively, and a statistical *t* test indicated the results were significantly different. At least part of the difference may be ascribed to loss of particulate matter in the exhaust ports of the shouldered actuators. Again, dose ratio was not affected by number of actuations for up to 170 actuations of the bioMDDs.

The effects of storage time and temperature were examined for Bti-filled PDCs that had been stored at room temperature (24 °C) or while refrigerated (4 °C) for periods of 9 to 25 months. After 9 months of storage, dose ratios were 65 and 71% for the samples stored at 24 and 4 °C, respectively. At the end of 25 months, dose ratios for the PDCs that had been refrigerated were an order of magnitude greater than those stored at room temperature. Refrigerated PDCs yielded dose ratios of 32% after storage for 1 year; therefore, a PDC filled with a Bti formulation could be expected to provide useful output after being stored for at least 1 year.

An unusual behavior was exhibited by the Bti-filled bioMMDs: after 9 months of storage, the Bti dose ratios were 65 and 71% for the room temperature and refrigerated samples, respectively. In contrast, the dose ratios of the Bti-filled devices tested with and without shouldered axial flow actuators (Figure 6) were 12.1 and 18.4%, respectively, after storage at room temperature for 3 months before use. We do not have a supportable explanation for this behavior; however, we are conducting additional experiments to determine whether the cause might be either the use of Bti powder in one set of experiments and a liquid suspension in the other, or the use of different ratios of vegetative cells or spores in the two experiments.

A set of experiments was conducted using BG spores to determine the performance of bioMDDs with no actuator, a hub-type actuator, or a nozzle actuator. Four replicate PDCs were tested for each type of actuator. An ANOVA statistical test showed significant variations between some of the actuation approaches and some of the replicates of each type of actuator. The average dose output of all test devices was 65 cfu/actuation, and the residual error was 24 cfu/actuation, for which the CV would be 24% for a randomly chosen bioMDD with one of

the three actuation approaches. Again, this suggests that the bioMDDs would not be applicable to instrument calibration. However, given that the output of any individual bioMDD was constant over the span of 170 actuations, it is possible that these devices are appropriate for use beyond functionality testing. A selected bioMDD could be checked in the laboratory against a detector or identifier with acceptable performance, and if the dose-to-dose variation was acceptable (such as exemplified by the 24% CV for the device used to acquire the data shown in Figure 7), that same bioMDD device could be used to check the performance of a detector or identifier in the field.

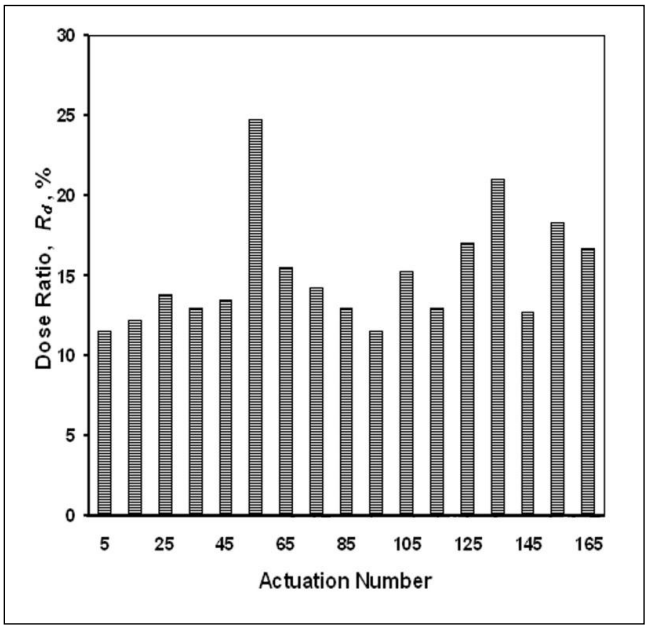

Figure 9. Constancy of Bti output from a bioMDD with no actuator. The average dose ratio was 15.1%.

4.5 Functionality Checks on UV Detectors

BioMDDs were used for functionality testing of two near-real-time UV bioaerosol detectors. Aerosol generated from one actuation of a bioMDD containing 1 µm fluorescently tagged PSLs, which had been prepared 3 years before the test, was sampled with a TSI UV-APS. The device not only successfully detected the PSLs, but also revealed that the dose ratio for the PDC was 16%. A second detector, which serves as a trigger for a collection-identification system that has a high level of power consumption, was tested with bioMDDs that had been filled with BG, γ-irradiation–killed BG, DNA from BG, Bti, ovalbumin, and 1, 2, 3, and 5 µm PSLs. The detector triggered after ingestion of BG, γ-irradiation–killed BG, DNA of BG, and some PSLs. Because the flow rate for air into the detector was 80 L/min, the samples may have been too dilute to have triggered alarms for all aerosols; however, more optimized doses would likely have resulted in complete alarming.

5. RECOMMENDATIONS

In general, bioMDDs should be suitable for testing functionality of bioaerosol detection and identification systems in the field, and perhaps could also be used to check particle size measurements, but not concentrations, of fielded size-measurement devices such as optical particle counters. The shelf life of the devices when filled with spore formulation should be about 1 year, provided the PDCs are refrigerated during storage. The shelf life of PSL-filled PDCs stored at room temperature is at least 9 months and could be considerably greater than 1 year. Also, if a PDC has been stored before use, it should be sonicated. Depending upon the application, either nozzle-type actuators that release a spray at a right angle to the PDC axis or hub-type actuators that release a spray in an axial manner should be acceptable.

A nominal 10 mL canister fitted with a 50 μL metering valve should provide up to 170 actuations; however, the bioMDD should be vigorously shaken and primed with at least two actuations before use. Because of the output constancy of individual bioMDDs with use (up to 170 actuations), it may be possible to use a selected laboratory-tested device to check the performance of a fielded detector or identifier; however, this approach would only be suitable for use in special cases, where the particular PDC could be reliably tracked, and a dose-to-dose variation on the order of 25% would be tolerable.

A comparison of the variability of shot weights for a bioMDD containing BG spores (Figure 3) with the variability of dose for either a bioMDD containing Bti spores (Figure 7) or PSL microspheres (Figure 4) reveals much greater variability in output when the formulation is a hydrosol. From the dose point of view, significant improvements could be made in bioMDD performance if particle retention within the devices were substantially reduced.

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ACRONYMS AND ABBREVIATIONS

AD	aerodynamic diameter
ANOVA	analysis of variance
APS	aerodynamic particle sizer
BA	*Bacillus anthracis*
BG	*Bacillus atrophaeus* var. *globigii*
bioMDD	bioaerosol metered dose distributor
Bti	*Bacillus thuringiensis* subsp. *israelensis*
cfu	colony-forming units
CV	coefficient of variation
HFA134a	1,1,1,2-tetrafluoroethane
PCR	polymerase chain reaction
PDC	pressurized dispensing container
pMDI	pressurized metered dose inhaler
PSL	polystyrene microsphere


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